REMARKS

Summary of the Invention

The invention features nucleic acid molecules encoding biofilaments that enable the expression and secretion of the biofilament in milk-producing or urine-producing cells of a mammal. The nucleic acid molecules of the invention can be used to generate transgenic embryos that, in turn, are used to generate the mature animals that produce the biofilament in the animals' milk or urine. In another aspect, the invention provides methods of producing biofilaments in cultured cells.

Summary of the Office Action

Claims 1-5 and 7-21 are pending in the case. Claim 2 stands rejected under 35 U.S.C. § 101. Claims 1-5 and 7-21 stand rejected under 35 U.S.C. § 112, first paragraph. Claims 5 and 13 stand rejected under 35 U.S.C. § 112, second paragraph. Each of the rejections is addressed below.

Rejection of Claim 2 under 35 U.S.C. §§ 101 and 112, first paragraph

The Embryo of Claim 2 has Utility

Claim 2 was rejected under 35 U.S.C. § 101 and 35 U.S.C. § 112, first paragraph, for alleged lack of utility. The Examiner contends that the claimed invention is not supported by either a specific asserted utility or a well-established utility, and thus one skilled in the art would not know how to use the invention. Applicants respectfully

disagree.

Claim 2 is drawn to a non-human mammalian embryo comprising a cell whose nucleus comprises the nucleic acid molecule of claim 1. The embryo of claim 2 clearly has a well-established utility, which is to generate the adult animal that produces the desired biofilaments. This utility is specifically asserted in the specification. For example, at page 2, lines 7-14, the specification states that the nucleic acid molecule included in the claimed embryo "enables secretion of the biofilament by the milk-producing cells or the urine-producing cells, into, respectively, milk or urine of the mammal that develops from the nucleic acid molecule-containing embryo."

However, even if the utility of the claimed embryo were not specifically described in the specification, the utility requirement has been met, because the claimed embryo has a well-established utility. "An invention has a well-established utility if (i) a person of ordinary skill in the art would immediately appreciate why the invention is useful based on the characteristics of the invention..., and (ii) the utility is specific, substantial and credible." MPEP 2107.02(II)(B). Applicants submit that a person of ordinary skill in the art would immediately recognize the utility of the claimed embryo for generating adult transgenic animals capable of expressing the desired biofilament proteins in their milk or urine. Applicants further submit that this utility is specific to the claimed subject matter, substantial in that it defines a "real-world" use, and credible.

In the Office Action dated January 4, 2001, the Examiner asserts that the embryos

are merely intermediates formed in producing a transgenic animal that has utility, but the embryos themselves do not have utility. While the Examiner is correct that the utility of the claimed embryos is to produce adult animals. Applicants respectfully disagree that this utility does not satisfy §101. In *In* re *Reiners v. Mehltretter*, 236 F.2d 418, 111 USPQ 97 (C.C.P.A. 1956), the predecessor to the Federal Circuit, the Court of Customs and Patent Appeals, held that "compounds employed as intermediates to produce other directly useful compounds [are] themselves useful" (Emphasis Added). Thus, the embryo is an intermediate of the animal and the biofilaments, just as is the claimed nucleic acid molecule (which was not rejected for lack of utility). Since the transgenic animals which develop from the claimed embryos and the biofilament proteins produced by the animals have utility, the embryos also have utility. Withdrawal of the rejections under 35 U.S.C. § 101 and 35 U.S.C. § 112, first paragraph, on the issue of utility is respectfully requested.

Rejections under 35 U.S.C. § 112, first paragraph

The Claimed Invention is Enabled by the Present Specification

Claims 1, 3-5, and 7-21 are rejected under 35 U.S.C. § 112, first paragraph, for an alleged lack of enablement. Applicants respectfully traverse this rejection.

Although a patent must contain a description that enables one skilled in the art to make and use the claimed invention, "[a]n inventor need not, however, explain every last detail since he is speaking to those skilled in the art." *DeGeorge v. Bernier*, 768 F.2d

1318, 226 USPQ 758 (Fed. Cir. 1985).

The specification of the above-captioned patent application contains ample guidance and specific instructions as to how to generate the claimed constructs and transgenic animals, and how to perform the claimed methods for producing a biofilament. The specification teaches, in sufficient and enabling detail, a person of ordinary skill in the art how to make and use the invention. Indeed, these very instructions were used successfully by the inventors to make and use the invention, as is explained in detail in the accompanying Declaration of one of the inventors, Costas Karatzas (a copy of which is enclosed for the Examiner's convenience).

Claims 1 and 7-12, Directed to a Nucleic Acid Molecule, are Enabled by the Specification

Claim 1 is directed to a nucleic acid molecule, comprising three elements, the first of which is "(i) a nucleic acid sequence encoding a biofilament." Examples of nucleic acids encoding a biofilament (*e.g.*, ADF-1, ADF-2, ADF-3, and ADF-4 spider silk genes) are given in Table I, page 10, and on page 9, line 11, through page 10, line 16, of the specification. Applicants further teach that "[a]ny of a variety of procedures well known in the art may be utilized to clone additional biofilament-encoding genes" (page 10, lines 17-21, of the specification), and go on to instruct that

> one such method for obtaining a biofilament-encoding gene sequence is to use an oligonucleotide probe generated by the

> Nephila clavipes spidroin 1 gene sequence...to screen an arachnid or insect cDNA or genomic DNA library for sequences which hybridize to the probe. (Page 10, line 22, through page 11, line 11, of the specification).

The second element of the nucleic acid of claim 1 is "(ii) a promoter that directs expression of a polypeptide in milk-producing cells or urine-producing cells, said promoter operably linked to said sequence." Applicants provide in the specification a detailed description of urine- and milk-specific promoters, signal sequences, and termination regions that can be used to construct the nucleic acid molecules of the invention. For example, Applicants state that:

> [e]ukaryotic expression vectors may be generated which drive the synthesis and secretion of proteins (e.g., biofilament proteins) in the milk or urine of an animal transgenic for a nucleic acid molecule...[These] expression cassette[s]...[consist] of elements necessary for proper transcription, translation, and secretion in the desired eukaryotic cell...Each expression vector will contain a signal sequence which directs the expressed gene product to be secreted from the mammary or uroepithelial cells. (Page 15, lines 13-15, page 16, lines 14-15, and page 18, lines 15-16, of the specification.)

In addition, the specification describes the use of specific, known promoters to make the constructs of the invention; these were known to be effective to make prior art transgenic animals, and the inventors used one of them, the whey acid protein (WAP) promoter, as described in the specification (page 22, line 16 to page 23, line 25 and Figure 1B), to successfully make animals of the invention, as described in the Karatzas Declaration. Other known promoters recited in the specification include the casein promoter and the

uroplakin II promoter, for specific expression of the biofilament protein in the milk or urine of the claimed transgenic animal (page 21, line 19, through page 24, line 12; Figures 1A and 1C).

The third element of claim 1 is "(iii) a leader sequence that enables secretion of said biofilament by said milk-producing cells or said urine-producing cells, into, milk or urine, respectively of a mammal." The specification teaches that many milk or urine specific promoters can be used with their signal sequences or with the silk and/or fibroin gene signal sequence. See page 16 lines 16-25. Additional enabling description of leader sequences is found at page 18, lines 14-24.

For all the above reasons, Applicants submit that claims 1 and 7-12 are fully enabled by the specification and that the rejection of these claims under 35 U.S.C. § 112, first paragraph, must be withdrawn.

Claims 2-5, Directed to Transgenic Mammals, are Enabled by the Specification

The use of the recited genes and promoters to make the claimed genetic constructs was clearly sufficient at the time the application was filed to allow a scientist of ordinary skill in the field to generate the claimed transgenic animals. The specification, on page 26, lines 10, through page 29, line 17, teaches, in enabling detail, how to use known methods to generate transgenic animals that express a biofilament in their milk or urine using the claimed and fully described constructs. For example, the specification teaches

that:

> transgenes can be introduced into embryonic stem cells (ES cells)...by electroporation, microinjection, or any other techniques used for the transfection of cells which are known to the skilled artisan...[Furthermore], ES cells can be used as a source of nucleic [acid molecules] for transplantation into an enucleated fertilized oocyte, thus giving rise to a transgenic animal. Page 26, line 21 to page 27, line 4, of the specification.

The methods used to make the transgenic animals are fully described, and further detail would not have been required by one of ordinary skill in the art. Indeed, the methods of transgenesis described within the specification were well known in the art and have been successfully used to produce transgenic animals.

The Examiner asserts that the "transgenic art" is "unpredictable" and therefore it would require "undue experimentation" to practice the invention. Applicants respectfully disagree. In fact, as Dr. Karatzas notes in his Declaration, at the time of the invention, generation of transgenic animals had become routine. Applicants again refer to the Karatzas Declaration to make another point: that the declaration supports a separate, independent reason for withdrawal of the rejection. Even if the generation of transgenic animals had been unpredictable at the time of the invention, in this instance the inventor's prediction, that following the instructions given in their specification would result in the successful production of a transgenic animal, <u>was</u> <u>accurate</u>. That workers attempting to make other classes of transgenic animals might have failed is not relevant to whether these animals could have been made: they could have been made, and they were made,

using precisely the methods described in the specification. The Karatzas Declaration describes the production of three founder goats transgenic for the spider silk ADF-3 gene, nine founder goats (six female, three male) transgenic for the spider silk MaSpI gene, and eleven founder goats transgenic for the spider silk MaSpII gene, all of which were generated using the methods described in the specification. Furthermore, high levels of biofilament protein were produced by these animals. Therefore, the invention, as described in the specification, works, and works well. Based on the reasons set forth above, Applicants respectfully request that the enablement rejection of these claims be withdrawn.

Claims 13-21, Directed to Methods for Producing a Biofilament, are Enabled

The Examiner alleges that the specification fails to provide an enabling disclosure for the preparation of the claimed species of transgenic animals because the guidance in the specification is limited to mice. The Examiner interprets the term "embryonal cell" recited in claim 13 to encompass both embyronal stem cells and fertilized oocytes. The Examiner asserts that neither the prior art nor the instant specification teaches how to transfect fertilized oocytes. The Examiner further alleges that while the prior art does teach how to transfect embryonal stem cells, such technology is limited to the mouse. Applicants respectfully disagree.

First, it was known in the art at the time the application was filed how to transfect fertilized oocytes. See, for example, Donehower et al. (U.S.Patent No. 5,569,824),

Brinster et al., *Cell* 27:223 (1981), Costantini et al., *Nature* 294:92 (1981), Harbers et al., *Nature* 293:540 (1981), Wagner et al., *Proc. Natl. Acad. Sci. U.S.A.* 78:5016 (1981), and Palmiter et al., *Science* 222:809 (1983). Second, at the time the application was filed, transfection of embryonal stem cells was not limited to mouse cells. It was known in the art that transgenic rabbits, sheeps, and pigs could also be produced by transfection of embryonal stem cells (see, for example, Hammer et al., *Nature* 315:680 (1985), Pursel et al., *J. Reprod. Fertil. Suppl.* 41:77 (1990), Rexroad et al., *Mol. Reprod. Dev.* 1:164 (1989), and Wieghart et al., *J. Reprod. Fertil. Suppl.* 41:89 (1990)). Therefore, based on the knowledge of one skilled in the art at the time the application was filed and the guidance provided in the specification, claims 13-21 are fully enabled.

Applicants also note that none of the grounds for rejection of the instant claims advanced by the Examiner are applicable to claim 14. Therefore, Applicants submit that claim 14, and claims dependent thereon, are allowable, and respectfully request the Examiner's acknowledgment thereof.

The Specification Contains a Written Description of the Invention of Claims 2-5

Claims 2-5 were rejected under 35 U.S.C. § 112, first paragraph, for an alleged lack of written description. The Examiner asserts that the claimed subject matter was not described in the specification in such a way as to reasonably convey to one skilled in the art that the inventors, at the time the application was filed, had possession of the claimed invention.

Specifically, the Examiner states that, because the specification contains no working examples, no transgenic animals having the claimed phenotype have been described. It is respectfully submitted that this rejection is in error and should be withdrawn.

The claimed phenotype of the mammals of the invention is the ability to produce a biofilament in their milk or urine. As is discussed above, and proven in the work described in the Karatzas declaration, the animals made by the methods described in the specification do in fact have precisely this phenotype. Further, the specification contains a clear, detailed, and accurate description of this phenotype. For example, on page 26, line 10 through page 29, line 17, the specification provides Example 8, which describes the generation of transgenic animals expressing biofilament(s) in milk or urine using methods to introduce transgenes into the pronuclei of fertilized oocytes or into embryonic stem cells (ES cells). The specification then describes how these cells are used to produce a transgenic animal (see page 26, line 10, through page 27, line 4, of the specification). The specification then states that these transgenic animals contain "the spider silk gene...subcloned into an expression vector that contains a casein gene promoter, such that expression of the spider silk gene is controlled by the casein gene promoter." The specification further describes biofilament production in milk using the whey acidic protein (WAP) promoter and biofilament production in urine "under the transcriptional control of the uroplakin promoter." (See page 27, lines 6-24, of the

specification.). It is submitted that this written description is completely sufficient to satisfy the written description requirement.

Claims 5 and 13 are Definite Under 35 U.S.C. § 112, Second Paragraph

Claims 5 and 13 were rejected under 35 U.S.C. § 112, second paragraph, as being indefinite. Specifically, claim 5 was rejected for the recitation of "said animal" because the phrase lacks antecedent basis. In response, claim 5 has been amended to replace the term "animal" with the term "mammal." Claim 13 was rejected for its recitation of "a biofilament encoding a nucleic acid." In response, the phrase "a biofilament encoding a nucleic acid" in claim 13 has been removed. Claim 13 was also alleged to be indefinite in the recitation of the phrase "a milk-producing or urine-producing cell derived from said transformed embryonal cell" because neither milk-producing nor urine-producing cells can be derived from an embryonal cell alone. In response, claim 13 has been amended to clarify that the milk-producing or urine-producing cells are found in the animal. Applicants respectfully submit that these amendments to the claims overcome the rejections based on 35 U.S.C. § 112, second paragraph.

CONCLUSION

Applicants submit that, in view of the above, all of the claims are in condition for allowance, and such action is respectfully requested. If there are any charges or any credits, please apply them to Deposit Account No. 03-2095.

Respectfully submitted,

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Marked version showing changes made

5. (Twice Amended) A non-human transgenic mammal in which the genome of cells that contribute to urine production in said mammal [animal] comprises the nucleic acid molecule of claim 1, wherein said promoter is urine-producing cell-specific, and wherein said mammal secretes said biofilament of claim 1.

13. (Twice Amended) A method for producing a biofilament, said method comprising the steps of:

(a) providing an animal comprising milk-producing or urine-producing cells that express and secrete said biofilament [embryonal cell transformed with a biofilament encoding a nucleic acid molecule that expresses and causes secretion of said biofilament from a milk-producing or urine-producing cell derived from said transformed embryonal cell]; and

(b) [growing said transformed embryonal cell to produce an animal comprising biofilament expressing and secreting cells; and

(c)] isolating said biofilament from said milk-producing or urine producing cells of [biofilament expressing and secreting cells from] said animal.

Claims as Pending [06632/011001]

1. A nucleic acid molecule comprising (i) a nucleic acid sequence encoding a biofilament, (ii) a promoter that directs expression of a polypeptide in milk-producing cells or urine-producing cells, said promoter operably linked to said sequence, and (iii) a leader sequence that enables secretion of said biofilament by said milk-producing cells or said urine-producing cells, into, milk or urine, respectively, of a mammal.

2. A non-human mammalian embryo comprising a cell whose nucleus comprises the nucleic acid molecule of claim 1.

3. A non-human female transgenic mammal in which the genome of the mammary tissue of said female mammal comprises the nucleic acid molecule of claim 1, wherein said promoter is milk-producing cell-specific, and wherein said mammal secretes said biofilament of claim 1.

4. The mammal of claim 3, wherein said mammal is a selected from the group consisting of a rodent, a ruminant, and a goat.

5. A non-human transgenic mammal in which the genome of cells that contribute to urine production in said mammal comprises the nucleic acid molecule of claim 1, wherein said promoter is urine-producing cell-specific, and wherein said mammal secretes said biofilament of claim 1.

7. The nucleic acid molecule of claim 1, wherein said biofilament is spider silk.

8. The nucleic acid molecule of claim 7, wherein said spider silk is dragline silk.

9. The nucleic acid molecule of claim 1, wherein said biofilament, when secreted such that the secretion is subjected to shear forces and mechanical extension, has a poly-alanine segment that undergoes a helix to a β-sheet transition, said transition forming a β-crystal that stabilizes the structure of said biofilament.

10. The nucleic acid molecule of claim 1, wherein said biofilament has an amorphous domain that forms a β-pleated sheet such that inter-β sheet spacings are between 3 angstroms and 8 angstroms in size.

11. The nucleic acid molecule of claim 1, wherein said biofilament has a C-terminal amino acid motif comprising an amorphous domain and a crystal forming domain, said motif having a sequence that is at least 50% identical to SEQ ID NO: 2.

12. The nucleic acid molecule of claim 1, wherein said biofilament has a consensus sequence that is at least 50% identical to SEQ ID NO: 3.

13. A method for producing a biofilament, said method comprising the steps of:

(a) providing an animal comprising milk-producing or urine-producing cells that express and secrete said biofilament; and

(b) isolating said biofilament from said milk-producing or urine producing cells of said animal.

14. A method for producing a biofilament, said method comprising the steps of:

(a) providing an animal cell transfected with a nucleic acid molecule that contains (i) a nucleic acid sequence encoding a biofilament, (ii) a promoter that directs expression of a polypeptide in an animal cell, wherein said promoter is operably linked to said nucleic acid sequence encoding said biofilament, and (iii) a leader sequence that causes secretion of said biofilament by said cell;

(b) culturing said transfected cell under conditions in which said biofilament is secreted into the culture medium of said cultured cell; and

(c) isolating said biofilament from said culture medium of said cultured transfected cell.

15. The method of claim 13 or 14, wherein said biofilament is spider silk.

16. The method of claim 15, wherein said spider silk is dragline silk.

17. The method of claim 13 or 14, wherein said biofilament, when secreted such that the secretion is subjected to shear forces and mechanical extension, has a poly-alanine segment that undergoes a helix to a β-sheet transition, said transition forming a β-crystal that stabilizes the structure of said biofilament.

18. The method of claim 13 or 14, wherein said biofilament has an amorphous domain that forms a β-pleated sheet such that inter-β sheet spacings are between 3 angstroms and 8 angstroms in size.

19. The method of claim 13 or 14, wherein said biofilament has a C-terminal amino acid motif comprising an amorphous domain and a crystal forming domain, said motif having a sequence that is at least 50% identical to SEQ ID NO: 2.

20. The method of claim 13 or 14, wherein said biofilament has a consensus sequence that is at least 50% identical to SEQ ID NO: 3.

21. The method of claim 13 or 14, wherein said animal is a mammal.